Cytotaxonomy of Sisyrinchium

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C Y T O T A X O N O M Y

O F

S I S Y R I N C H I U M

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INTRODUCTION

The Iridaceous genus of Sisyrinchium is found throughout North and South America. It appears to be a fairly large genus, some estimates giving the total number of species as about 250, but much confusion exists in the literature about the species classification, and many of the names are synonymous with previously described ones.

Different members of the genus have various forms and flower colours, but there exists a basic plan, and there are basic features of the vegetative and floral structures. The stems, which may be simple or branched to give peduncles, are frequently flattened and winged, although sometimes terete; similarly the leaves are usually sword-shaped and two-edged, but occasionally these also may be terete. In most species the flowers are borne in a simple umbel at the apex of the stem. This inflorescence is protected by two leaf-like bracts, which are called the spathe, and the flowers emerge from between them borne on fine pedicels, and open one at a time. The floral parts are in multiples of three. The perianth is of six similar or fairly similar segments, united at the base. The stamens have their filaments united for varying distances at the base, forming a tube round the trifid style. The fruit is a tripartite capsule, with axile placentation, and usually containing many seeds.

A part of the confusion of nomenclature which has been mentioned concerns the identity of the first Linnean species: Sisyrinchium Bermudiana. Two taxa, originally described under this single name, have since been separated by botanists into two distinct species. Much has been written on the probable correct identity of these two forms, and part of the work for this thesis has consisted of studying this problem and reading the literature on the subject.

It has been mentioned that most of the species of this genus occur in parts of North and South America. However one form has been found growing, apparently naturally, in areas of suth-west Ireland. A preliminary study has been made of the problems of identity and origin of this plant.

Practical work has been carried out on those various species of which it has been possible to obtain seed or plants. The studies to be described include some general biology, cytology and species relationships within the genus.

Descriptions of the plants worked on, and sources of the material, are given in the appendix.

CHAPTER I

SISYRINCHIUM BERMUDIANA Linn.

In Sisyrinchium, as in many other plant genera, the names given by Linnaeus are taken as the starting point in modern classification. Linnaeus may not have had quite the same concept of 'species' and 'genus' as we have, but it is necessary, when using Linnean names, to follow his usage as closely as we can. In some cases this is simple, as in S. palmifolium Linn. (described in his Mantissa 1767). There is sometimes, however, difficulty in identifying the actual form to which Linnaeus was referring in his brief and often incomplete descriptions. On these occasions the Linnean Herbarium, at the Linnean Society of London, may show the actual plant referred to by Linnaeus.

The original description of Sisyrinchium Bermudiana given by Linnaeus (1764) is very brief and incomplete. He gives two varieties, α from Virginia and β from Bermuda. There is considerable morphological difference between these two forms, as is shown in the diagrams (Fig. 1); α variety is under a foot high, with a simple, slender and very narrowly

(a) (b)

Fig.1 Sisyrinchium from (a) Virginia and (b) Bermuda.

winged stem which is terminated by a spathe, with two very unequal bracts, and only two flowers. The β variety, from Bermuda, is twice the size in all its parts, with a broadly winged and branching stem, and many flowers in a spathe.

Linnaeus gives references to Plukenet (1696) and Dillenius (1744) for each of his varieties; and also botanists after Linnaeus have considered the two as distinct species, by reason of morphology, size and habitat. As a result of this division into two species, much discussion has taken place as to which of the two should retain the name given by Linnaeus.

The references quoted by Linnaeus (Plukenet 1696 and Dillenius 1744) show clearly how he derived the name Sisyrinchium Bermudiana. Bermudiana was the generic name used by Dillenius in his polynomial descriptions, and similarly Sisyrinchium was that used by Plukenet. Linnaeus, in making this binomial for this species, chose to honour these two previous workers. Therefore the specific name of Bermudiana is not used as a geographic adjective referring to the place of origin of the species, but as a historic reference. It is unfortunate that the β variety which Linnaeus describes should come from Bermuda, but this does not prevent his Virginian variety α from being the primary form described.

In view of the confusion which has existed about these two varieties, I examined the specimen of Sisyrinchium Bermudiana in the Linnean Herbarium. In the photograph of the original plant (Fig. 2) this will be seen to have the slender unbranched form and unequal bracts typical of the Virginian species. It has been suggested by Hemsley (1884) that Linnaeus may never have seen the Bermudan plant, since he had no specimen of it in his Herbarium. However, there is an unlabelled specimen of a larger form of Sisyrinchium in the Herbarium which might possibly have been this. The only other species he describes is S. palmifolium of which there are several clearly labelled sheets. (The form of the Bermudan plant is best illustrated by Redouté (1807) in his Liliaceae.)

Miller was the first botanist after Linnaeus to consider the two forms as separate species. Unfortunately he interpreted the name of S. Bermudiana as referring primarily to the Bermudan plant, and described the Virginian plant under the new name of

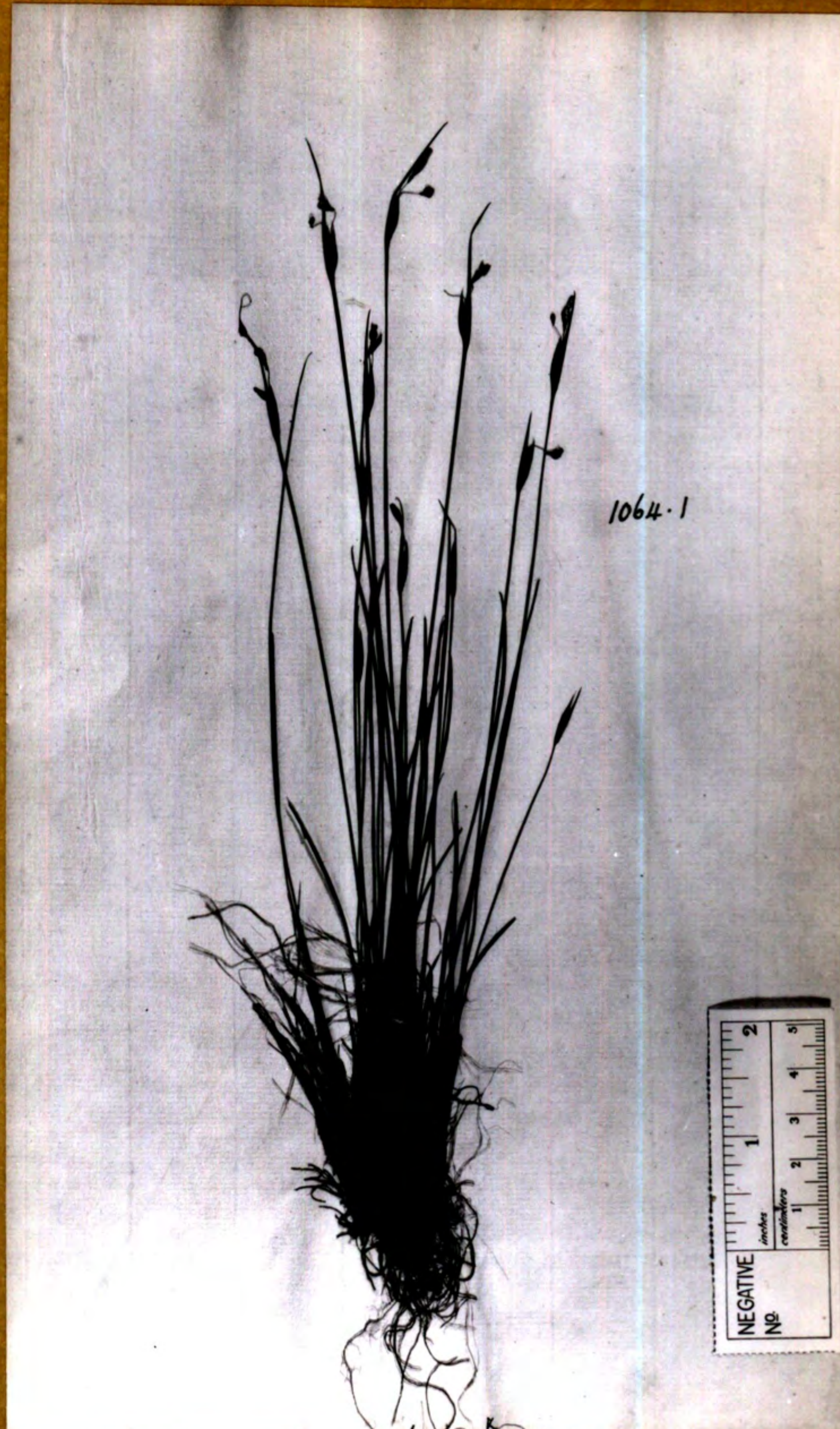

Fig. 2 Sisyrinchium Bermudiana

(From the Linnean Herbarium)

S.angustifolium (1768). Most botanists (e.g. Hemsley, Bicknell and Fernald) have followed Miller's example and retained the Linnean name for the Bermudan plant, though they have varied greatly in their choice of name for the original Virginian species. They have ignored the facts to which I have drawn attention, calling them "an undue insistence on merely technical points, at best of uncertain bearing in this particular case" (Bicknell 1896). Hemsley casually writes: "I have not taken the trouble to turn up every book in which the two species are likely to be mentioned". He even suggests that the "error" of the name _S.Bermudiana_ being applied to the Virginian species may have been caused by the Bermudan plant having disappeared from English gardens while the name was retained for the Virginian species. Those who have trouble to study Linnaeus' sources and specimens have held very different views. Morong (1893), Farwell (1917) and Shinners (1957) have all discussed this problem, and in each case concluded that the name of _Sisyrinchium Bermudiana_ should be retained for the Virginian plant, because Linnaeus had designated it as var. α, and in any event had derived the name from previous ones without implying locality of origin.

It has been observed by Farwell (1917) that if Linnaeus had intended the specific name to denote the place of origin, he would have called the plant S.bermudiense. This latter ending and the small first letter were actually used by Plukenet in his polynomial description of the Bermudan plant, and as such quoted by Linnaeus describing his β variety. Some authors have misquoted the name as being S.bermudianum (Cézard 1958), thus furthering the illusion of its being an adjectival specific name, and this is often used to describe the garden form Sisyrinchium.

Watson (1889) appears to associate the origin of the name S.Bermudiana with Tournefort: "the Tournefortian Bermudiana from Bermuda", thus quoting a reference not mentioned by Linnaeus, describing and illustrating what appears to be a different plant, with a floral formula of K_6 C_6 (Tournefort 1719). He misses the significance of the references to Dillenius and Plukenet by which Linnaeus explains the actual origin of the name.

Since the name S.Bermudiana was believed by many authors to refer to the Bermudan plant, that is Linnaeus' variety β, they described the Virginian plant by several new names. Miller describes it first in 1759 and again, with the new name of <u>S.angustifolium</u> Mill., in 1768. Cavanilles (1788) described a plant with "simple two-edged stem, often leafless; spathe longer than the flowers" which he called <u>S.anceps</u> Cav., and amongst the synonymy he gives <u>S.Bermudiana</u> Linn. (var. α). He illustrates a plant with one branched and one simple stem, which may or may not be the same as <u>S.Bermudiana</u>. Michaux (1803) also gave a new name, <u>S.mucronatum</u>, to a plant with "very narrow leaves; scape setaceous, simple". He also refers to the spathe as "amethyst-coloured", a feature still visible in the Linnean Herbarium specimen. Michaux gives the habitat of this plant as Pennsylvania, and also describes a separate species, <u>S.Bermudiana</u> Linn., with "scape spread to form two wings, all the branches at the end". He gives the habitat of this species as Pennsylvania to Carolina andquotes <u>S.anceps</u> as a variety. Lamarck (1783) gives the name <u>S.gramineum</u> Lam. for the simple stemmed species. Asa Gray (1865), in the fourth edition of his Manual of Botany, gives two varieties of <u>S.Bermudiana</u> in North America:

var. <u>anceps</u>: broadly winged scape var. <u>mucronatum</u>: slender and narrowly winged scape.

In the sixth edition (1890) he describes two separate species:

<u>S. angustifolium</u>: stem simple, spathe solitary and terminal

<u>S. anceps</u>: scape usually branched, bearing two or more peduncled spathes.

It is unfortunate that more notice has been taken of the one branched stem illustrated by Cavanilles than of his unbranched stem, or of his description. Fernald (1946) followed Gray in considering the name to refer to a branched form, in spite of the original description which clearly stated that it was "simple, usually leafless". Fernald takes <u>S. mucronatum</u> as the valid name for the simple, slender Virginian species.

Bicknell (1899) describes <u>S. angustifolium</u> as having "pedicels erect or nearly so" in addition to an unbranched form. This Fernald describes as a "standard misconception of what Miller had as <u>S. angustifolium</u>, Lamarck had as <u>S. gramineum</u>, and Cavanilles as <u>S. anceps</u>". The plant of the Linnean Herbarium has pedicels distinctly reflexed to the capsules. This might be due to the drying and pressing of the specimen, but since in other forms the pedicels have been observed to be reflexed, it is considered more likely to be natural.

Miller, in his Latin description of S.angustifolium, refers to "pedunculis longioribus" than in the previously described S.Bermudiana and in the English discussion describes the stems as "very slender terminated by two pale blue flowers .... standing upon longer foot-stalks.. ." Miller quite clearly refers to the pedicels and in no way implies that the main stem branches to give peduncles. Fernald (1946), however, puts great emphasis on the words "pedunculis longioribus" and interprets Miller's description as of a plant with "long peduncles and .... flowers on 'longer' footstalks". It is hard to know how he justifies two independent translations of Miller's word "pedunculis", thus giving his plant both peduncles and pedicels. As a result of this misinterpretation Fernald derives a plant with a branched stem.

We thus have the following interpretations of this name:-

S.angustifolium Mill. (S.Bermudiana Linn.)

S.angustifolium sensu Bicknell (with erect pedicels)

S.angustifolium sensu Fernald (with branched stem)

Michaux, as has been mentioned, gave the name S.mucronatum to the simple-stemmed slender plant (habitat Pennsylvania in this case) and described S.Bermudiana as having "scape spread to form two wings, all the branches at the end".

Retaining Linnaeus' name for the simple-stemmed plant of the Eastern States, the synonymy appears to be:-

S.Bermudiana Linn.

S.angustifolium Mill.

S.mucronatum Michx.

S.gramineum Lam.

(S.anceps Cav. possibly)

The name S.Bermudiana has also been given to the Bermudan plant which is now called S.iridiodes Curtis (1790) and to the branched species of the Eastern States. This may be S.anceps Cav., and also the garden plant often called S.Bermudianum.

The plant described by Bicknell as S.angustifolium is said by Fernald to be S.montanum Greene.

It is beyond the scope of the present study to trace all the descriptions of the various species allied to, and at times confused with, _S.Bermudiana_ Linn. It does however seem quite conclusive that, despite the converse views of many botanists, Linnaeus gave the name _S.Bermudiana_ primarily to the species from Virginia, as illustrated in the photograph of his specimen.

CHAPTER II

DIFFERENCES BETWEEN SECTIONS BERMUDIANA AND ECHTHRONEMA

The experimental work in this thesis had the object of trying to characterise the taxonomic position of the available plants of the genus, and particularly that of the Irish variety, which appears to be distinct from S.Bermudiana. This was attempted on a morphological study of two of the four sections of the genus, coupled, where possible, with a study of the chromosome number and morphology, and with the results of hybridization between the various species available for this thesis.

The genus has been divided into four sections by Bentham and Hooker (1883) and Engler and Prantl (1890), which they described as Bermudiana, Echthronema, Eriphilema, and Nuno. The work described in this thesis has been carried out almost entirely on the first two sections.

The most obvious difference between the species of these two groups lies in the flower colour; the Bermudiana have a blue flower, often with a yellow centre;

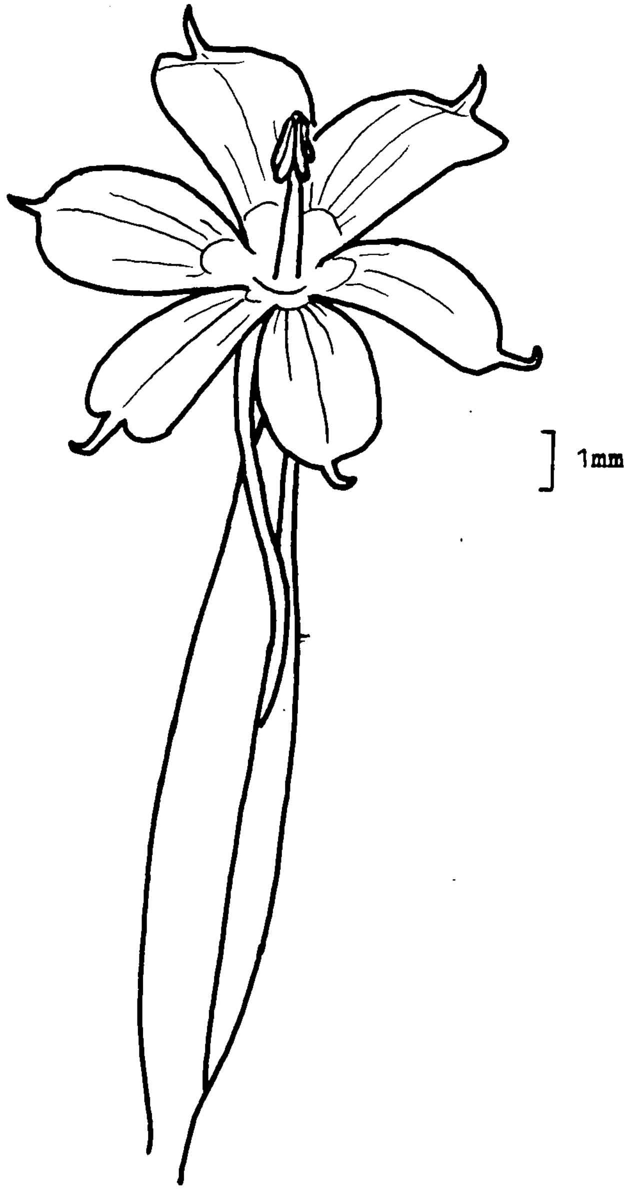

Fig. 3 "Sisyrinchium hibernicum" (10)

the _Echthronema_ have an all-yellow flower. The perianths of the blue flowers have typically a mucronate tip to the segments, whilst those of the yellow flowers are usually obtuse. There is also a difference in the appearance of the foliage, which is referred to by Bicknell (1900) as "an indefinable foliar attribute which separates" the two sections even "apart from the flowers". It may be the result of slight differences in the proportions, colour and glaucousness of the leaves and stems.

Differences in the structure of the organs in typical flowers of the two sections will be described in detail during the discussion on pollination mechanisms, and may be seen from the diagrams of plants 10 and S15 (Figs. 3 and 4). There is a great difference in the shape of the capsules of the few species examined (Fig. 5), possibly due to the difference in form of the placenta. In the _Echthronema_ it runs along most of the central axis, but in the _Bermudiana_ it is limited to a small, slightly swollen central area.

Differences of chromosome number, shape, and also of intensity of staining by Feulgen's method will be described. In all the blue-flowered species recorded

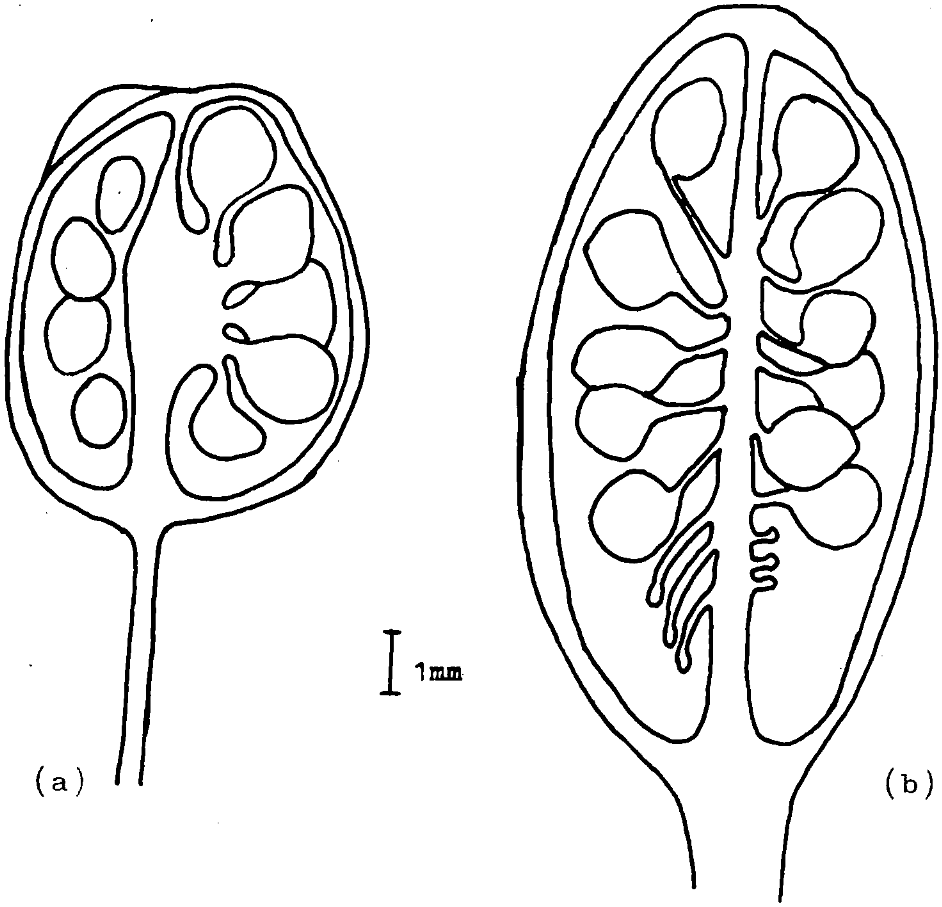

Fig. 5 Capsules of (a) section Bermudiana (10) and (b) section Echthronema (S4)

the basic chromosome number was 8 and in the yellow-flowered ones 9.

A wider survey of the range of variation in the two sections must be made before the natural limits of the genus Sisyrinchium can be ascertained. In view of the differences to be discussed, the division suggested by Salisbury (1812) and Bicknell (1900) may be justified. Salisbury recommended, on the basis of morphology, that Echthronema be treated as a separate genus, for which he suggested the name of Hydastylus. It would then be necessary to examine such apparently distantly related species as S. striatum Smith (Fig. 6) in the light of the full range of variability of the section and determine the taxonomic position of this species.

The remaining two sections of the genus, namely Eriphilema and Nuno, might also be considered as distinct genera, but the present work can offer no contribution to this view.

CHAPTER III

CHROMOSOME STUDIES

A study was made of the chromosome numbers of many of the available forms of *Sisyrinchium*. Most counts were made on root-tip material. During the early part of the year, because of insufficient numbers of cell divisions, the older roots were trimmed to promote more young growth; a few plants did not recover from this treatment. In about February to March natural root growth was resumed and sufficient divisions were then found. Root tips were collected at about mid-day and pre-treated with paradichlor benzene for one to two hours. The technique of cytological investigations is that of Darlington and La Cour (1947). Acetic alcohol was generally used as a fixative, but occasionally Carnoy's fixative was employed. The root-tips were stained in bulk by Feulgen's method. Generally the optimum time was 10-12 minutes for blue-flowered species and about 20 minutes for yellow ones. It was often impossible to obtain as good contrast in the latter as in the blue-flowered forms. *S.striatum* and *S.odoratissimum* were much easier to stain clearly than most species and the hydrolysis time for these did not appear so critical. For the former, a test series, with hydrolysis times from about 4-20 minutes, showed reasonable staining, especially after the longer times.

Squash preparations were made of the root-tips, mounting the slides in 'Euparal'. Study of chromosomes at meiosis was attempted in several species, but it was found difficult to obtain preparations of chromosomes at suitable stages of cell division. Lamps giving long day-length (about 15 hours) were used in the laboratory in an attempt to induce earlier flowering and increasing the opportunity of studying good preparations. No conclusive results were obtained.

When fixing the whole inflorescence at one time it was found difficult to obtain the correct stage from the relatively few flowers. Therefore a method was developed for removing only one stamen from a flower bud at a time, thus making the greatest use of the limited material available. The enclosing bracts were slit

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The work was carried out in order to secure a longer flowering period and not designed as a scientific experiment on light-induction. As a result of this, the presence of so many variables prevented any general conclusions from being drawn concerning the flowering times of any of the plants.

lengthwise and held apart whilst a bud of the right stage was selected and a single anther removed with a mounted needle, leaving the rest of the flower intact. The bracts were then put back in position and the rest of the inflorescence protected from drying by a small polythene bag, made from strips of polythene about 1 cm x 6 cm, heat-sealed at the side and end. By this method the remaning two anthers, as well as the other buds in the inflorescence, were able to develop further, increasing the chance of finding the required stage of meiosis.

P.M.C. preparations were made by the standard aceto-carmine squash method, after fixing in Bradley's fixative. These cells were found very much easier to squash than the root-tip preparations and satisfactory dispersion of the chromosomes was obtained.

Drawings were made of the best cells in preparation of both mitosis and meiosis, usually with the aid of camera-lucida, but occasionally freehand. Counts were then made from these drawings.

Great variation in the number, size and shape of the chromosomes was found in the different species of <u>Sisyrinchium</u> examined. Examples of these different numbers and types of chromosomes are shown in the drawings.

Table I shows the chromosome counts obtained from the various species examined. In all those with a low chromosome number, represented by stock numbers 8 (S. striatum, Fig.7a) and 17 (S. odoratissimum, Lindle, Fig. 7b) and S52, the diploid number is 18, with only one cell counted with less. The studies on meiosis support this finding, all 12 cells examined giving n = 9 (Fig.8). In both the species examined where meiotic as well as mitotic preparations were available, the chromosome number was easily counted in the former type. The problems of analysis of cells at mitosis increased with increasing number of the chromosomes, and the preparation of cells with sufficient spreading of the very many small ones for all to be distinguishable was very difficult.

In those species of both the sections Bermudiana and Echthronema with counts mainly between 30 and 38 the distribution of counts is a little wider. However, interpretation of these results is still possible, and S4 (probably S. brachypus Bickn. or S. Californicum Aiton) shows 7 cells with 2n = 36, and the number is probably

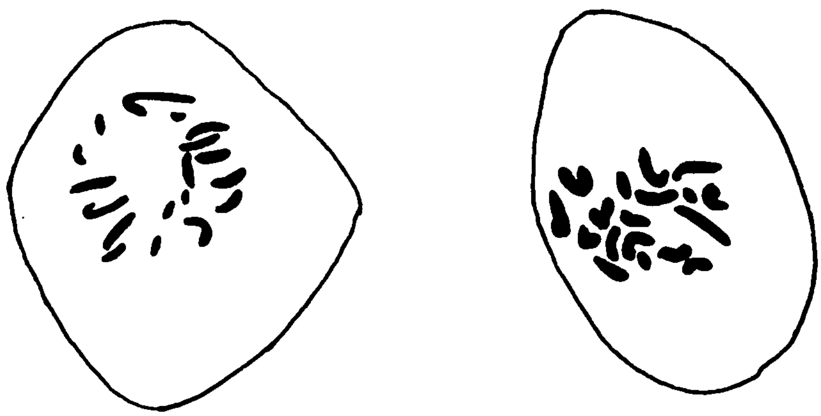


Fig. 7 Mitosis in (a) S.striatum (2n=18) and (b) S. odoratissimum (2n=18) (approx:1600 X)

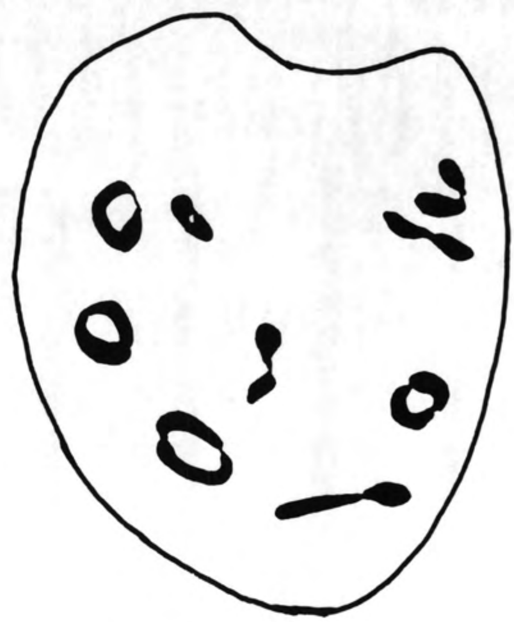


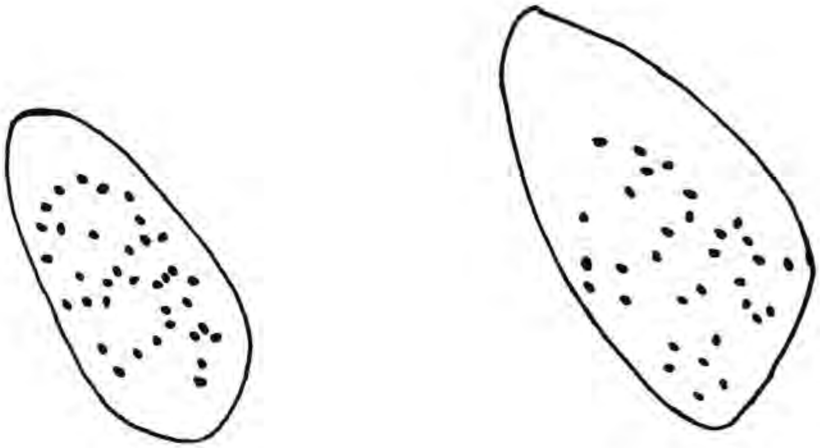
Fig. 8 Meiotic Chromosomes of

S. striatum (9 $\overline{\underline{II}}$)

(approx: 1600 X)

the same for S15 (S. convolutum Nocca, Fig. 9a). In the species from the section Bermudiana in this group, the counts are less decisive but indicate a lower mean. 2n = 32 (Fig. 9b) has been reported by Bowden (1945), Löve and Löve (1958) and Bøcher and Larsen (1950) for a number of species (Table II) and the same number is possibly correct for both S11 (Fig. 9b) and S54 (S. bellum Watson).

Those members of the section Bermudiana with a very high chromosome number posed the greatest problem in making chromosome counts. In stock 12, which is the common blue-flowered garden plant often erroneously called S. Bermudianum, many cells were counted, but the range of numbers obtained is very large. In many cases the variation in results is most likely to be due to some of the chromosomes being obscured, and the correct number is therefore probably in the range 90-98 (Fig. 10). Counts of 2n = 96 have been reported by Bowden (1945), Bøcher and Larsen (1950) and Löve and Löve (1958), and it is possible that the same number is present in this species. Only a very few cells were available from S1 (S. gramineum Lam) and S2 (S. Idahoense Bickn), but these may also be found to have the same number on



(a) (b)

Fig. 9 Mitosis (a) <u>S. convolutum</u> (2n=36)
(b) <u>S. sp.</u> (S11) (2n=32)
(approx: 1600 X)

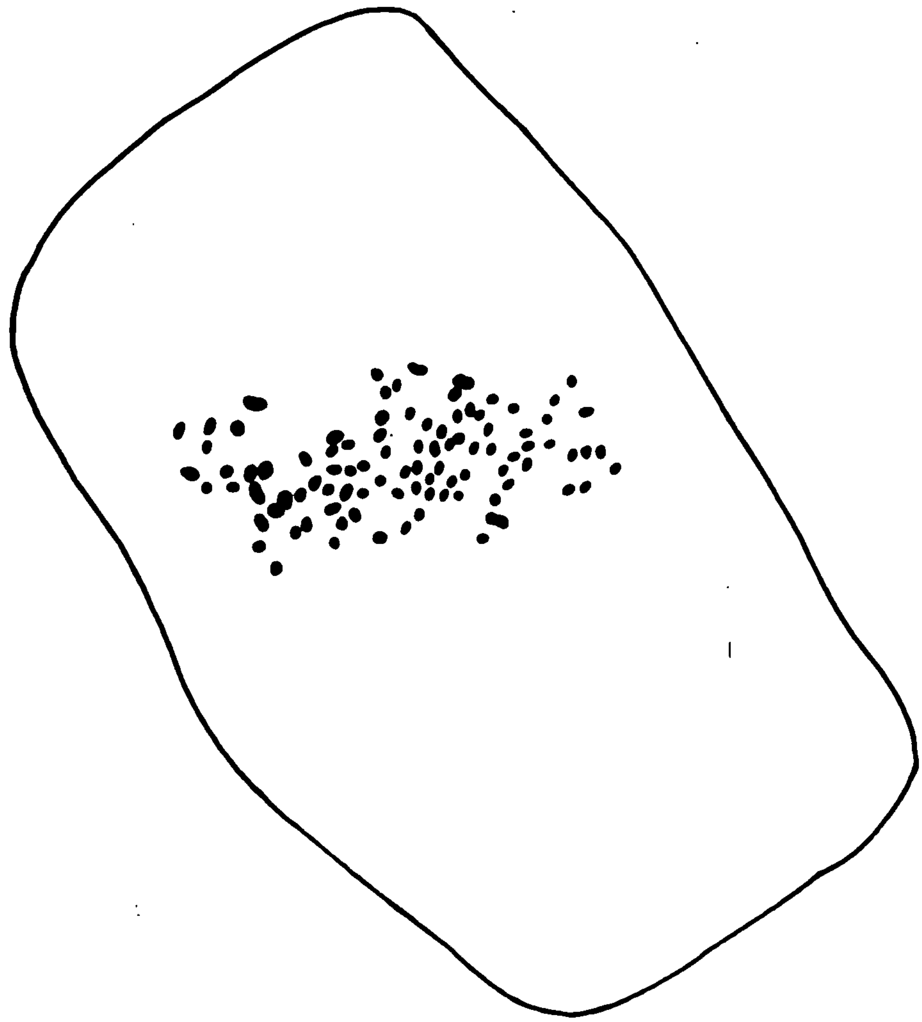


Fig. 10 Mitosis in a member of the section <u>Bermudiana</u> (12) 2n=96 (approx. 1600 X.)

further examination. Unfortunately preparations of meiosis were not available for these species.

The cells at mitosis available from the specimens of Sisyrinchium from Ireland give no indication of the chromosome number, but fortunately meiotic preparations were available from these plants. Some of the latter cells (Fig. 12) were very clear, and a count of n = 44 was obtained. In three of the clearest cells, where the chromosomal configuration could be analysed with some confidence, two showed 43 bivalent and 1 univalent, and one showed 43 bivalent and 1 trivalent. These counts, which suggest a diploid number of 2n = 88 (Fig. 11), do not agree with those of Löve and Löve (1958). Other aspects of this problem will be discussed during the chapter on Sisyrinchium in Ireland, but it is possible that both of the counts were made on hybrid plants, which would explain both the variation in number and the univalents and trivalent obscured at meiosis.

The counts of other authors, supported where possible by my own counts (Table II), indicate a difference in basic number between the two sections studied. In the section Bermudiana, all the counts recorded are based on the number of x = 8, and

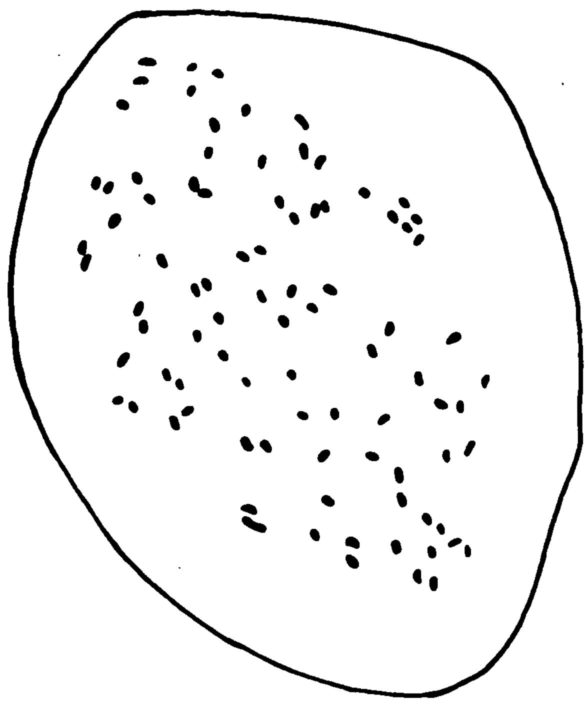


Fig. 11 Mitosis in "S. hibernicum" (2n=88)
(approx: 1600 X)

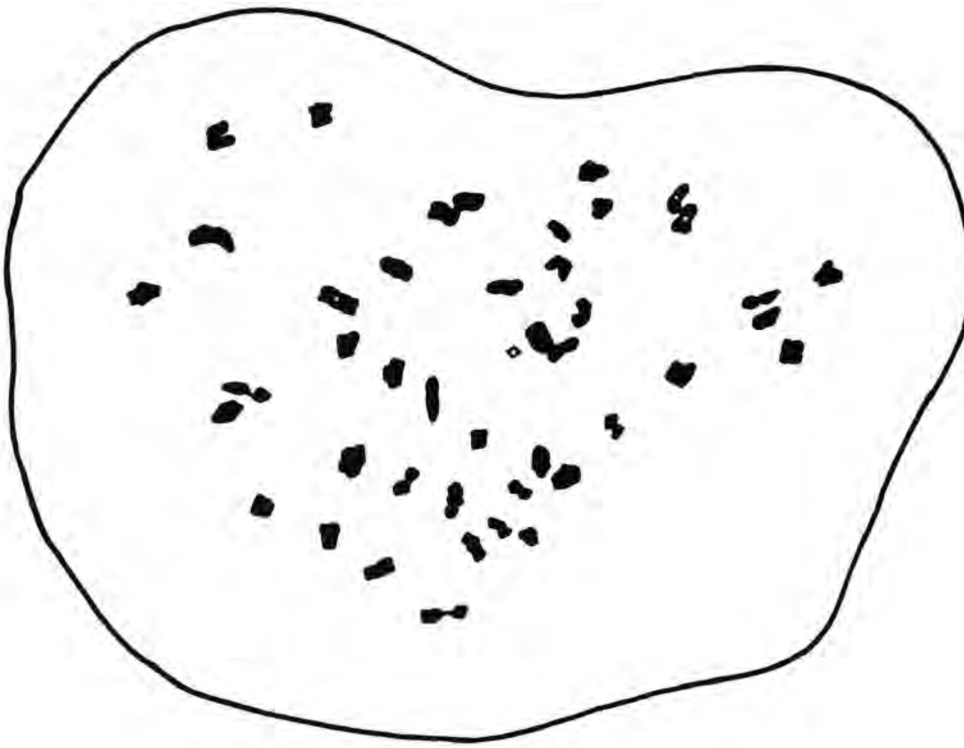


Fig. 12 Meiosis in "S. hibernicum" (43 II & 1 I)
(approx: 1600 X)

similarly in the Echthronema all are based on x = 9. Insufficient species have yet been counted in either section to prove this theory, but it is possible that the division into separate genera suggested by Salisbury (1812) and Bicknell (1900) on taxonomic grounds may be justified by the methods of cytotaxonomy.

TABLE I

CHROMOSOME COUNTS MADE ON SISYRINCHIUM SPP.

Stock Used	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
8 (2n)		1	3																						
" (n x 2)			12																						
17			3																						
S.52	3				1																				
S.4																2	3	1	1	2	7	2			
S.15																	1		1	2	2		1	1	
S.11												1			1		3	1	2						
S.54												1			2	1									

Stock Used	<80	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	>100
12	3	3	1		1	3	1	1	4	1	1	2	4	3	3		2	2	3	2		1	1
S.1										1									1				
S.2														1						1			1
10 (2n)	6			1				1	1	1						1			1				
* (n x 2)						1		1		7		2											

TABLE II

CHROMOSOME NUMBERS IN SISYRINCHIUM

Section *Bermudiana*

2n = 16	*S.sp*	Bowden (1945)	Chile
2n = 32	*S.bellum*	Bowden (1945)	N.America
	"	J.M.N. (S54)	
	S.montanum	Löve & Löve (1958)	N.America
	S.sp (*S.iridifolium*?)	J.M.N. (S11)	
	S.albidum	Löve & Löve (1958)	N.America
	S.labidum	Bowden (1945)	W.Indies
	S.mucronatum	Löve & Löve (.958)	N.America
	S.montanum	Bøcher & Larsen (1950)	Greenland
2n = 64	*S.sp*	Bowden (1945)	Chile
2n = 66	*S.sp*	Löve & Löve (1958)	Ireland
2n = 88	*S.sp*	J.M.N. (10)	Ireland
2n = 96	*S.angustifolium*	Bowden (1945)	N.America
	S.angustifolium	Bøcher & Larsen (1950)	(from Copenhagen B.G.)
	S.atlanticum	Löve & Löve (1958)	N.America

	S.montanum	Bøcher & Larsen (1950)	(from Copenhagen B.G.)
	S.Idahoense	J.M.N. (4, S2, S41)	
	"S.Bermudianum"	J.M.N. (5, 7, 9)	(garden form)
	S.graminifolium	J.M.N. (S1)	

Section Echthronema

2n = 18	S.stbiatum	Vilmorin	Chile
	S.striatum	J.M.N. (16)	
	S.iridifolium	Bowden (1945)	Brazil, Chile
	S.sp (chilense ?)	J.M.N. (S52)	
	S.macrocarpum	Covas	Argentine
2n = 34	S.Ca ifornicum	Maude (1940)	Ireland
2n = 36	S. Californicum	Bowden (1945)	California
	S.brachypus	Bowden (1945)	California
	S.convolutum	J.M.N. (S15)	
2n = 90 (approx)	S.sp	Bowden (1945)	California

Section Nuno

2n = 18	S.odoratissimum	J.M.N. (17)	

CHAPTER IV

SISYRINCHIUM IN IRELAND

A small group of plants in the British Isles constitutes what is called the American element in the British flora. These species are found in a few localities in the British Isles, but otherwise are either natives of North America or have their nearest relatives there. There has been much discussion on the origins of these plants. A few may have been introduced, either intentionally or otherwise, but some are thought to be natural.

Sisyrinchium is one of the members of this American element, occurring mainly in a number of counties in Ireland (Fig. 13), but it has also been found in a few localities in England and Scotland. The origin of the plants found in the latter is probably different from that of most of those in Ireland. All plants, however, of the blue-flowered type whether growing in England, Scotland or Ireland have previously been referred to as S. angustifolium Mill. It has already been shown that this species should be called S. Bermudiana Linn., and in this discussion, where the typical Virginian plant of Linnaeus is indicated, this latter name will be used.

The specimens from England and Scotland preserved in the herbaria of the British Museum and of the Royal Botanic Gardens, Kew, are similar to S. Bermudiana of North America; they have the same slender habit, narrowly winged stem and few small blue flowers. These plants are probably derived from garden escapes, as S. Bermudiana is often grown as a garden plant in this country.

The origin of the plants found in Ireland seems rather more complicated. In addition to some specimens similar to S. Bermudiana, a different form is also found, with larger, branched habit and many flowers per spathe. It has been suggested by Preager (1934) and Löve and Löve (1958) that this latter form may be indigenous to Ireland. This view is supported by the fact that the localities where it is often found are remote, and by the apparent stability of the populations, without the spreading usually associated with introduced plants. The localities from which it has been reported in Ireland are shown in the map.

In its most typical form, this plant is easily distinguished from S. Bermudiana and has a number of

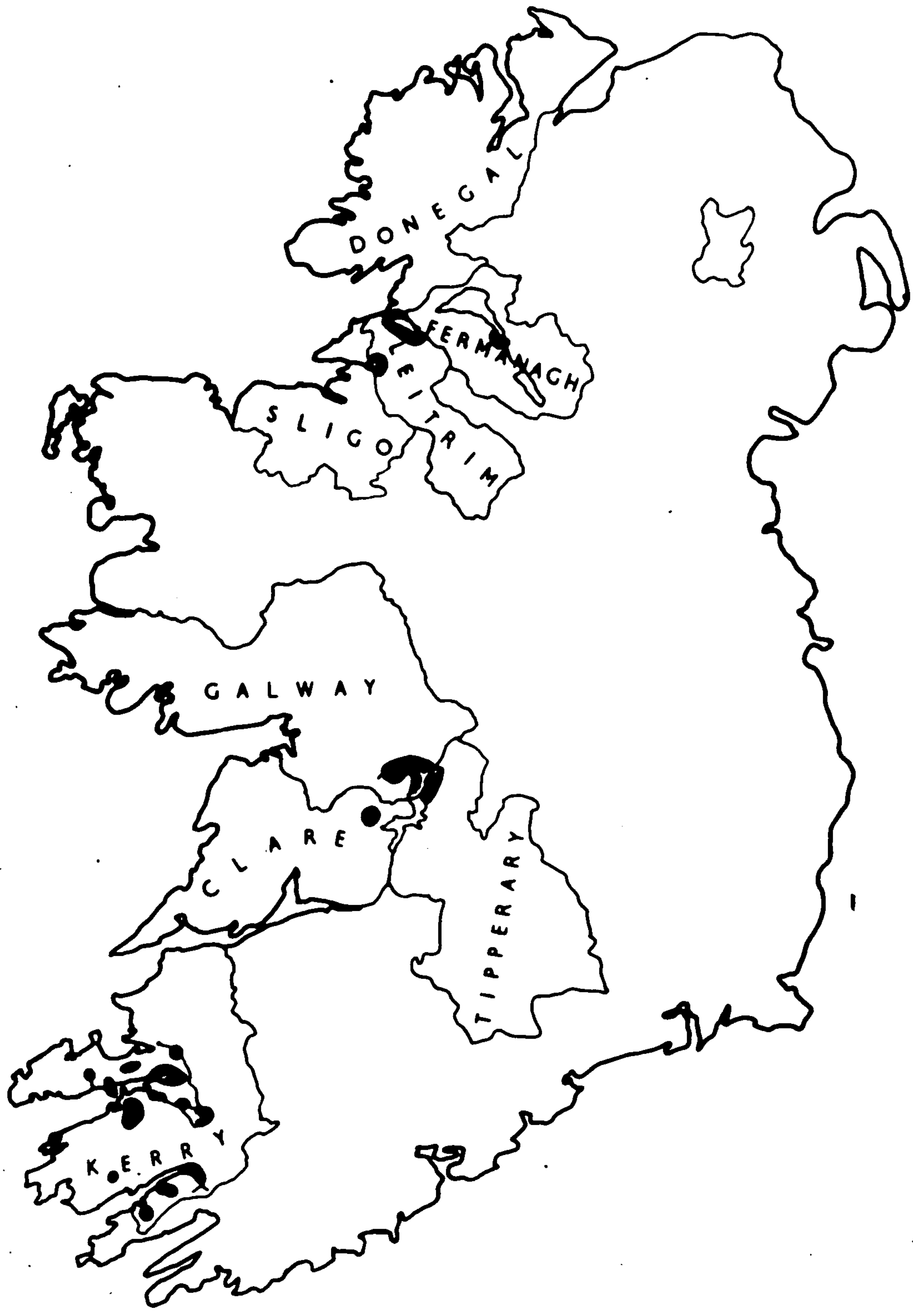


Fig. 13. Distribution of Sisyrinchium in Ireland.

sword-shaped radical leaves and a broadly winged stem. It may be up to 2ft high with a single leaf a little over half way up the stem, where it gives rise to two branches. Both the main part of the stem and the leaves are 3-4 mm wide, the branches being narrower and unequal in length. In a few cases there may be no stem leaf, or alternatively more than one, or a leaf may subtend 1-3 branches. At the apex of each branch is a spathe, protected by two bracts, the outer of which is longer than the inner. Each flower has six equal petals, which are bright blue with a yellow base. There are usually 4-8 flowers to a spathe, each open for only a few hours. The position of the point of branching on the stem varies with different populations. In the present work the branching index defined as the ratio :-

$$\frac{\text{length of longest branch}}{\text{total height of plant}}$$

has been used as a metric character of this point. Thus the lower the point of branching, the higher is the branching index, until in a plant with no apparent branch but only a simple stem the branching index is unity. In the typical Irish non-<u>Bermudiana</u> described above, it

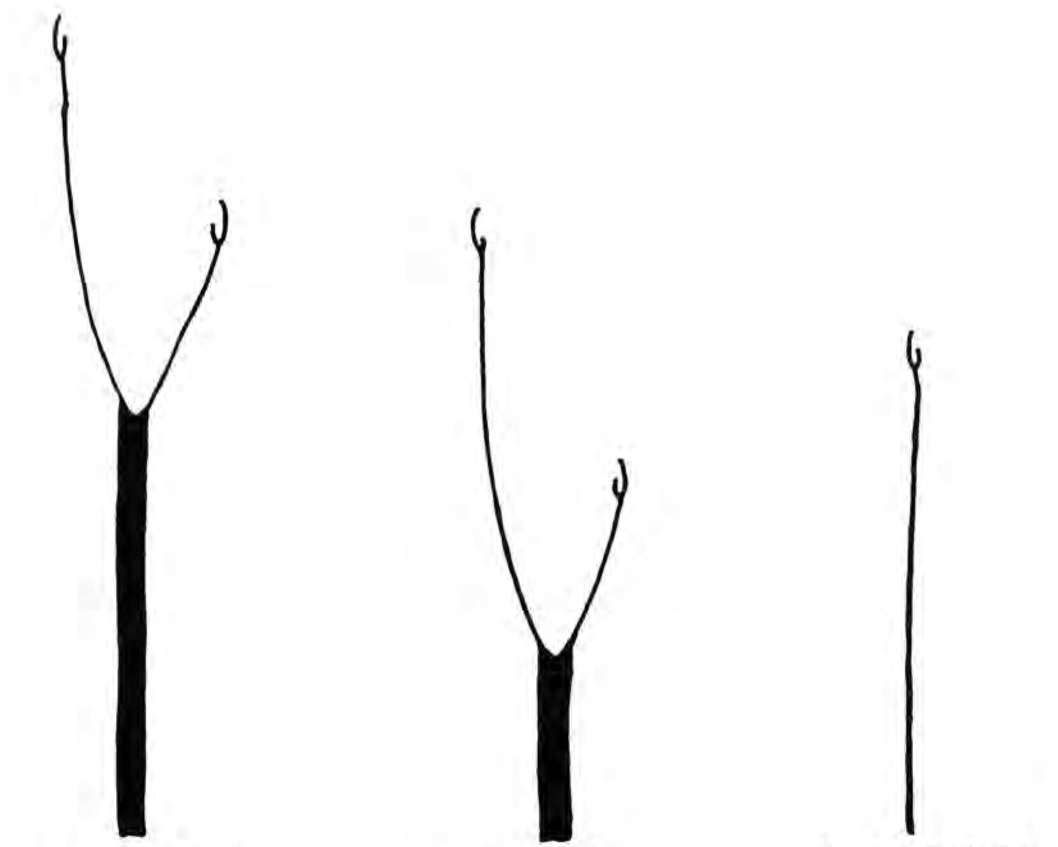


Fig. 14. Forms of Branching in Sisyrinchium from Ireland.

is usually between 0.3 and 0.4. For convenience I shall refer to this species as "*S.hibernicum*" (Fig. 14).

The herbarium material of *Sisyrinchium* from Ireland at the British Museum and Kew Gardens shows plants mainly from Co. Kerry and Galway, and only the situation in these two counties will be discussed here. Measurements in most cases are from the herbarium specimens.

In Co. Kerry the range of variation in the 15 plants measured is very limited, all being the typical Irish plant "*Sisyrinchium hibernicum*". The 24 plants examined from Galway were considerably more variable in the characters examined than those in Co. Kerry. The figures shown in Table III, a and b, represent measurements made on herbarium material, mainly from the British Museum and Kew Gardens. An analysis of the difference between the means for length of the main stem (below branch), total height (including branch) and branching index was carried out, using Student's 't' test (Geigy 1956)*. There was a

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* Formulae used:-

$$t = \frac{\bar{x} - \bar{x}'}{sD}$$

$$sD = \frac{s^2 x,x'}{N} + \frac{s^2 x,x'}{N}$$

$$s^2 x,x' = \frac{S(x^2) - \bar{x}S(x) + S(x'^2) - \bar{x}'S(x')}{N + N - 2}$$

(Geigy 1956)

significant difference between the means for both main stem ($t = 2.06$, $P < 0.05$, df $= 37.$) and total height ($t = 0.684$, $P = 0.05$, df $= 37.$). The most striking difference, however, was between branching indices ($t = 2.54$, $P = 0.02 - 0.01$, df $= 37.$).

this was P=0.5.

To show that there is a difference, not only between the average forms of the plants in the two populations but also between the amount of variation to be found within each of these two populations, Fisher's test for the comparison of variations (Brownlee 1949)* was used. The difference in variation between the lengths of main stem was significant ($F = 2.32$, $P = 0.05$, df = 14 and 23.), and again the significance of the difference observable between the range of branching index was very high ($F = 10.4$, $P < 0.001$, df = 14 and 23.).

The counts of numbers of flowers per spathe is included for interest, since there appeared to be a difference between pure "<u>S.hibernicum</u>" and the hybrid unbranched type. However, a statistical analysis

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*Formulae used:

$$F = \frac{s^2 \text{ (larger)}}{s^2 \text{ (smaller)}} \qquad \text{(Brownlee 1949)}$$

$$s^2 = \frac{1}{N-1}(S(x^2) - \bar{x}S(x)) \qquad \text{(Geigy 1956)}$$

was not considered justified on such a small sample, owing to the unreliability of these figures, as no flowers had emerged from the spathes in some specimens. The specimens preserved in the British Museum and at Kew which have been examined were collected from five localities in Co. Kerry but only from the one apparently large population in the Woodford area of Galway. The greater variation in the Galway specimens cannot therefore be explained by Sewall Wright effect acting on a number of small isolated populations.

It would appear from these figures that whilst the Sisyrinchium found in Co. Kerry may be considered as representing a uniform population, here described as "S.hibernicum", the plants from Galway are only partly of the same form. The unbranched plants from the latter county have the slender, short stem and floral and spathe characters typical of S.Bermudiana, native of North America. It might be tentatively suggested that some plants of S.Bermudiana have escaped in the Woodford area of Galway and resulted in hybridisation with the native "S.hibernicum" already present.

It is interesting to observe that what appears to be the hybrid form, intermediate between pure "S.hibernicum" with a branch and pure S.Bermudiana with a simple stem, has a branch arising from lower down the stem (Fig. 10). No plants were found with a higher point of branching than that normal in "S. hibernicum". A morphological comparison of the stem and branch in these unbranched plants with the stem and branch of the branched plants appears to indicate that the stem of the former resembles the branch, rather than the stem of the branched plants. The main stem of the branched plants is stouter and has a much broader wing. It has unfortunately not been possible owing to lack of time to examine the vascular anatomy of these stems and branches, but this might prove informative.

It would be interesting to cross artificially the two pure forms of Sisyrinchium and compare the branching index with the naturally occurring intermediate, and also to examine the fertility of the hybrid. On the evidence available from both morphology and apparently natural hybridisation, it would appear that the simple stem may be more accurately considered as a branch arising directly from the rootstock. This form of plant would be expected to give a short main stem as in the hybrid plant rather than a longer one (Fig. 14).

Only two chromosme counts are available for the Co. Kerry populations and none for Galway. Löve and Löve (1958) have reported a count of 2n = 64 from the Cloonee Loughs, and I have found 2n = c88 from Lough Caragh. No deductions can yet be attempted from these strange figures, and it will be necessary to make counts from many localities in all the counties in western Ireland where the plants grow before they can confidently be interpreted.

From the limited work it has been possible to do on the Sisyrinchium found in Ireland, it appears best to consider it as a form distinct from the North American S.Bermudiana, with which it has previously been associated. Further work must be done before it can be decided whether the plant here called "S.hibernicum" should be associated with or made a subspecies of any other North American species, or whether it can justifiably be described as a new species.

From comparison with herbarium material and descriptions of species from North America, "S.hibernicum" appears to be most similar to S.graminoides Bickn. However, in view of the large number of closely related species distinguished by Bicknell, this tentative suggestion requires verifying. A close comparison between these two forms from America and Ireland, including chromosome and hybridisation, would be illuminating. It is certainly distinct from S.iridiorides Curtis of Bermuda which is another branched form and often referred to as S.Bermudiana.

A third form of Sisyrinchium, S.Californicum, has been reported (Maude 1940) growing in parts of Ireland. This is presumed to be an introduced plant, identical with that found in California. The difference between the chromosome number reported by Maude and that of Bowden (1945) (Table II) is more likely to be an error in counting than an actual difference between the Irish and American forms of the species.

TABLE IIIa

SISYRINCHIUM FROM CO. KERRY

	Length main stem in cms.	Total height in cms.	Branching Index	No. flowers
	20	30	0.33	4
	20	36	0.42	5
	21	39	0.46	8
	20	31	0.35	5
	30	40	0.25	6
	25	40	0.37	5
	10	19	0.47	3
	14	23	0.39	4
	17	27	0.37	4
	24	39	0.38	6
	20	35	0.43	6
	28	41	0.32	5
	25	45	0.44	6
	20	30	0.33	5
	20	34	0.41	4
Mean	21.0	33.9	0.38	5.07

TABLE IIIb

SISYRINCHIUM FROM GALWAY

Length main stem in cms.	Total height in cms.	Branching Index	No. flowers
26	42	0.38	3
22	36	0.39	3
20	35	0.43	4
6	17	0.65	2
8	28	0.71	2
12	22	0.45	5
21	33	0.36	4
20	34	0.41	4
28	44	0.36	4
7	24	0.71	2
25	37	0.32	3
22	32	0.31	3
12	22	0.45	-
10	22	0.55	4
20	28	0.29	3
20	30	0.33	4
20	32	0.38	4

Length main stem in cms.	Total height in cms.	Branching Index	No. flowers
6	14	0.57	-
10	20	0.50	3
12	12	1.00	2
6	22	0.73	4
5	25	0.80	4
30	42	0.29	3
17	17	1.00	1
Mean 16.0	27.9	0.52	3.23

CHAPTER V

METHOD OF POLLINATION

In the genus Sisyrinchium the floral parts occur in multiples of three. There are six perianth parts, in two whorls of three, which may or may not differ slightly in size or shape. There are three stamens, with their filaments joined to form a tube round the style, the extent of fusion depending on the species. The style branches into three, each branch bearing a stigmatic tip.

Two basic modifications of the arrangement of the stamens and stigmas have been found in the species examined and are associated with a difference in flower colour, that is, each of the two sections studied has a characteristic arrangement. It appears from observations on these species that the method of fertilization depends on the relative arrangement of these organs.

In the blue-flowered species examined (Fig. 3), which are members of the section Bermudiana, the filaments are fused throughout their length, and the anthers are erect and close to the style (Fig. 15b). The branches of the style just surpass the anthers, as shown in the diagram, and are very slender. They consist of long

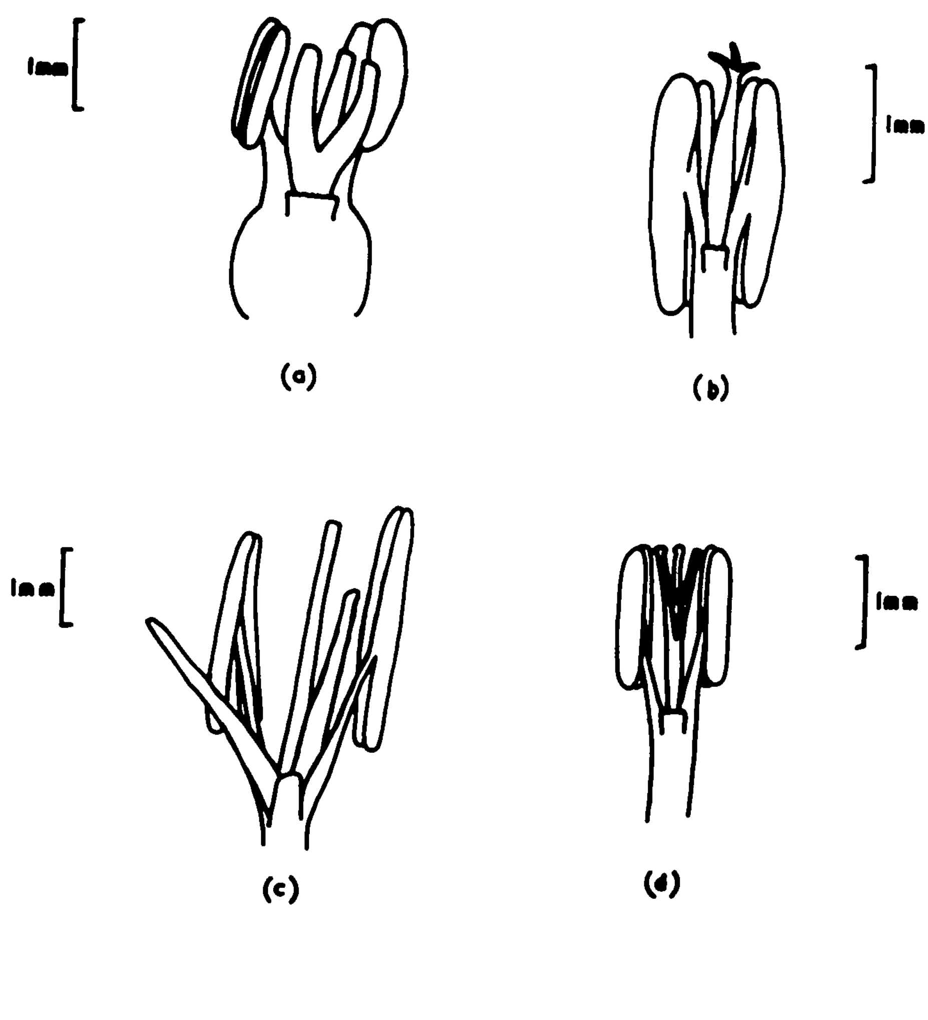

Fig. 15. Arrangement of stamens and style in *Sisyrinchium*.

narrow cells, with no rough or otherwise modified surface visible to indicate the stigmatic area (Fig. 16b). However, with careful treatment, by covering the styles with pollen and removing the excess with a sable brush, it is possible to show that the inner surfaces of these branches are more adhesive to pollen grains than the outer. The nature of the specialised surface is unknown, but it must be regarded as constituting the stigma.

When the flower of a species in this group opens, the stamens have already dehisced and liberated pollen over the outer surfaces of the style branches. These branches are, however, adpressed at this stage, protecting the stigmas. When the style-branches diverge, the flower is only open for one day, and often only a few hours. If cross-pollination is not brought about during this short period, self-pollination takes place, because as the petals wither the flower closes and the stamens are forced into contact with the now exposed stigmas.

In the yellow-flowered species examined (section <u>Echthronema</u>), the filaments are only fused at the very base. Beyond this short tube they diverge, bearing

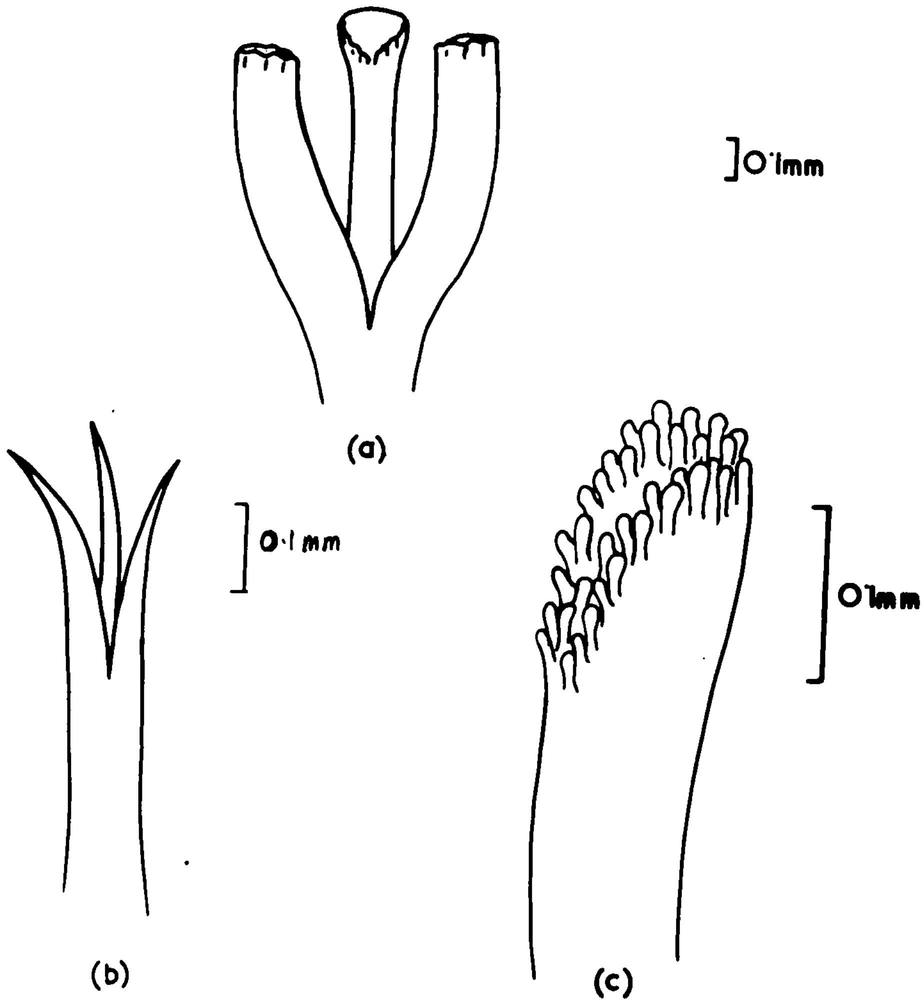

Fig. 16. Stigmatic surfaces in <u>Sisyrinchium</u>

long versatile anthers at their apices (Fig. 15c). The style branches into three at the end of the anther tube and the end of each spreading branch bears a stigma, the surface of which is covered with small papillae (Fig. 16c). The anthers dehisce when the flower opens, but are farther from the stigmas than is the case in the blue-flowered species, resulting in a lower frequency of self-fertilization. As in the section Bermudiana, withering of the petals may lead to self-pollination (if the flower has not been previously cross-pollinated), but the efficiency of this method appears to depend on the relative arrangement of the stamens and styles.

The frequency of self-pollination has been examined in a number of plants in an insect-proof greenhouse, avoiding any artificial transfer of pollen. It was found that in those plants with anthers close to the styles, as in the blue-flowered species, self-fertilization occurred in most cases, giving capsules full of seed. However, in those yellow-flowered species in which stamens and style branches diverge, as in S.convolutum, the capsules either aborted or contained only a few seeds. In S.striatum, with smaller and less divergent stamens and styles than in the other yellow-flowered species (S.brachypus, Fig. 17).,most capsules contained a normal amount of seed after self-pollination. As a result of this frequent selfing, it is possible that some species may form partly inbreeding populations, but this would require careful investigation before formulating any theory.

Owing to this arrangement of stamens and styles, great care is required in the artificial crossing of some species to avoid accidental selfing. In the yellow-flowered species it is possible to remove the anthers after the flower opens, and crossing is then quite a simple matter. In the blue-flowered species, however, it is necessary to remove the anthers before they dehisce. This must be done by cutting off the petals of the flower the morning it emerges from the bracts, before opening, and the anthers may then be removed with forceps, taking care to avoid causing premature dehiscence.

The table (Fig. 18) shows the results of the crossing experiments as indicated by seed set. Further work on the hybrids has not been possible, but certain facts are illustrated.

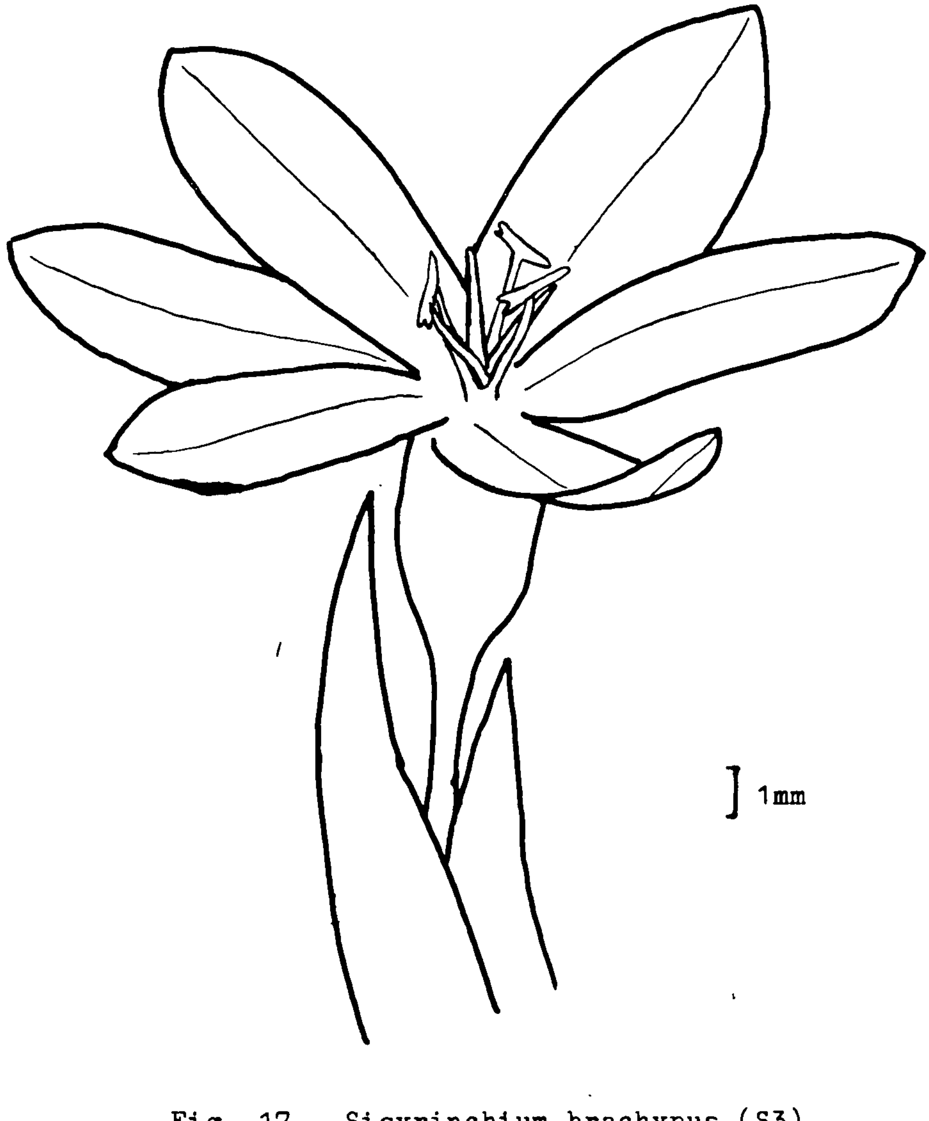

Fig. 17. Sisyrinchium brachypus (S3).

♀ \ ♂		blue flowered						yellow flowered				
		4	5	9	10	14	S11	1	S3	S4	S13	S15
blue flowered	4				/2							
	5	3/		2/	3/1		/2					
	9		2/		1/1		/2					
	10	/3	1/									
	14		2/	1/								
	S11		/1		/3	/2			/2			
yellow flowered	1										2/	/3
	S3										5/	
	S4								1/			2/6
	S13							2/		1/		/1
	S15								/1	/6	/5	

Fig. 18. Results of hybridisation in Sisyrinchium

Number of capsules: seed formed/aborted.

In both blue-flowered and yellow-flowered species, the closer the relationship between species, as shown by general appearance, the higher is the rate of seed-set. Very closely related blue forms were most successful, but no hybrids were obtained from stock S11 (Fig. 19), in spite of 10 attempts. Amongst other blue-flowered species, 15 were successful out of 22 attempts. In the yellow forms the findings are similar, with fewer successful crosses between apparently distant species, for example, only 2 successful crosses with S.convolutum (S15) and 22 unsuccessful ones. Variation in number of seeds per capsule is to be found in many cases, but generally there are fewer from crosses between less closely related forms.

It appears that, as in other outbreeding populations, it may be possible to obtain much interesting information in this genus by hybridisation experiments. This work has, of necessity, only been a preliminary study of the method of crossing in Sisyrinchium, and of the possibility of work on this genus, with its various sections and critical species. It is now desirable to study the hybridisation between all available species.

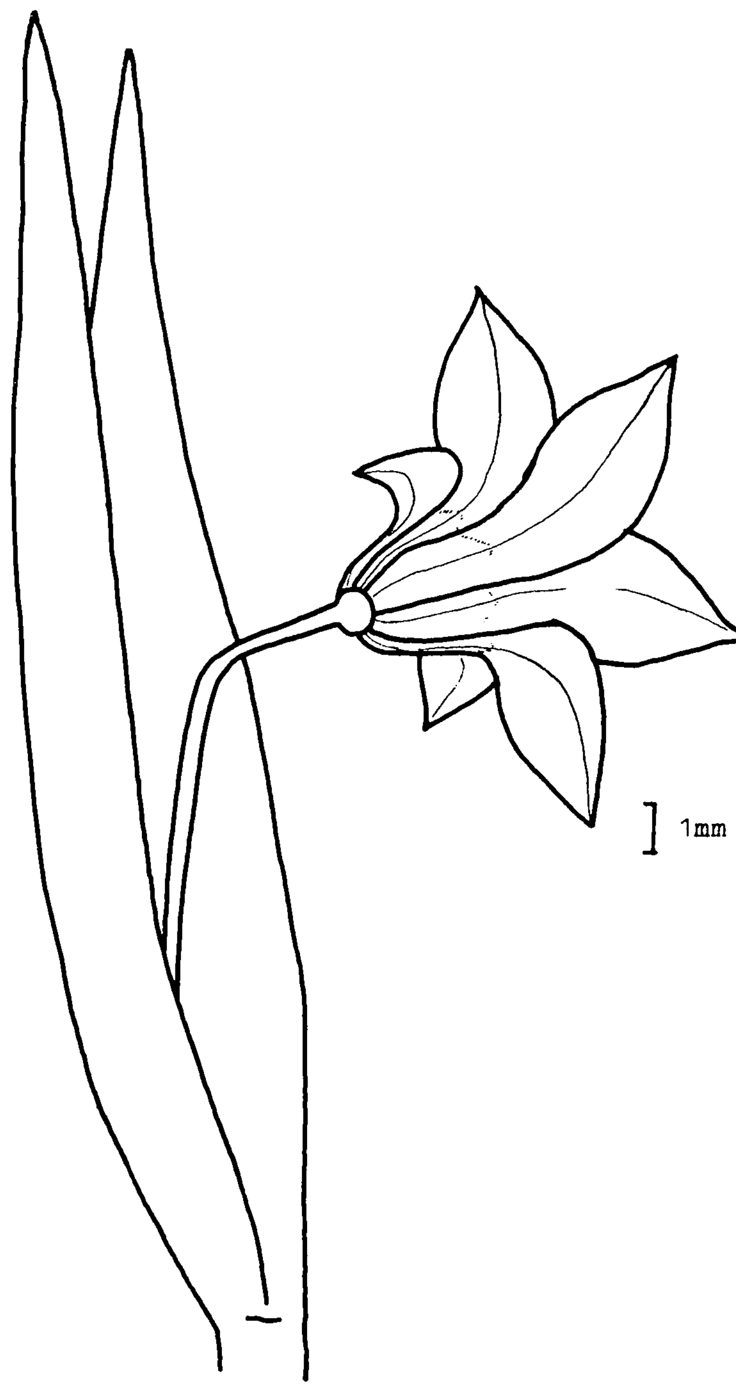

Fig. 19. Sisyrinchium species (S11).

CONCLUSION

Preliminary work was undertaken on various aspects of *Sisyrinchium*, to see if this genus provides suitable material for study by the methods of experimental and cyto-taxonomy. A number of problems were studied and the work carried out has been described.

The validity of two sections described by Engler and Prantl (1891) as *Bermudiana* and *Echthronema* has been examined. Particularly because of the difference in basic chromosome number (*Bermudiana* x = 8, *Echthronema* x = 9) they might be better considered as two separate genera, a suggestion made by Salisbury (1812) on the grounds of morphology. To justify finally such separation, further crossing experiments and the examination of the resulting hybrids are required, as well as more extensive chromosme counts.

The various identities of the species first described by Linnaeus have been examined; it has also been concluded on the available evidence that the name *Sisyrinchium Bermudiana* Linn. must be used to describe the Virginian species, often called *S.angustifolium* (Linnaeus variety α).

A form of *Sisyrinchium* grows in parts of Ireland

and is a member of what is called the "American element in the British flora". These plants appear in their natural form to be distinct from the S.Bermudiana of Virginia, with which they have been previously identified. Chromosome counts of 2n = 64 (Löve and Löve) and 2n = 88 (the author) do not solve the problem. Further work must be done on both comparative morphology and cytology before the question raised by Löve and Löve (1958) as to whether it should be considered a new species indigenous to western Ireland, or identified with a North American one, can be satisfactorily decided.

As has been shown in this thesis, there are several problems in the genus Sisyrinchium which are suitable for study by the methods of cytology and hybridisation. The work described has only been a preliminary investigation of the subject but has served to show that further investigation along the several lines described would be of interest and is required to settle many outstanding points of taxonomy and origin of this widespread and difficult genus.

APPENDIX

SOURCES AND DESCRIPTION OF THE STOCKS OF SISYRINCHIUM EXAMINED AND REFERRED TO IN THE THESIS

Section: BERMUDIANA

4; 5; 9; 14; Stem often simple. Rich blue flowers, large, with widely spreading mucronate segments. Filaments joined almost throughout length.
(St Andrew's Botanic Garden)

10; Stem branched, winged, about 3-4 mm wide. Flowers, smaller, light blue, shape as above. Pedicels reflexed in fruit. Stamens as above.
(From Co. Kerry, Ireland) (See Figs. 11, 12b, 13b)

S11; Stem branched, leafy, slightly jointed. Campanulate flowers with yellow centre, segment apices pale blue, acuminate. Bracts equal. Capsule smooth, globose. Filament tube swollen, anthers and stigmas different from corresponding
(Royal Bdanic Gardens, Kew) (Figs. 12a, 13a)

Section: ECHTHRONEMA

1; 2; S3; S4; Tall, stem unbranched, broadly winged. Leaves light green, staining paper purple on drying, often lax. Bracts usually unequal. Petals oblong, apex obtuse, yellow, brown veined on drying. Capsule oblong.

(1 & 2, St Andrew's Botanic Gardens; S3 & S4 Edinburgh Botanic Gardens) (Fig. 15)

18; S13; Similar to above but short, up to about 6". May be a variety of the same species. In both forms filament tube short, apex of filaments and style branches spreading, anthers versatile.

(18, Cambridge Botanic Gardens; S13, Royal Botanic Gardens, Kew)

S15 (S. convolutum); Stem branched, jointed, leafy. Flowers yellow, segments broad, obtuse, reflexed. Stamens and styles as above. Capsule obovate.

(Royal Botanic Gardens, Kew) (Figs. 14, 12c, 13c.)

8 (S. striatum); Tall, unbranched, broad stem. Leaves broadly lancelolate. Many spathes up main stem. Many yellow flowers per spathe. Perianth campanulate at base, segments slighty mucronate, yellow with brown purple veins. Stamens and styles smaller than above and more similar to members of the section Bermudiana.

(St Andrew's Botanic Gardens) (?S52) (Figs. 16, 12d)

Section: NUNO

17 (S. odoratissimum); Leaves terete, no flowers produced.

(Cambridge Botanic Gardens)

POST-SCRIPT

Since the draft of this thesis was completed, Löve and Löve (1961) have published the description of an Irish plant they call <u>S.hibernicum</u>. This is described as an unbranched plant, with 2n = 64, and will be seen to be different from the one here referred to, for convenience, as "<u>S.hibernicum</u>". The plant was not given a firm specific name in the thesis owing to the variation in both appearance and chromosome number in the forms described. It now appears even more desirable for a careful investigation to be made of the members of the genus in Ireland.

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